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1) Evans GF, 1994, J Lipid res, 35(9): 1634-45.
2) Schodel, F, 1992, J Virol, 66(1): 106-14.
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4) Roy, P, 1996, J Lipid Res, 37(1): 22-34.
5) Thomas, AP, 1996, Hybridoma, 15(5): 359-64.
Thank you.
Minh-Tam Davis
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could not use this ref. for 102 because it is not known how CETP is conjugated to Keyhole limpet hemocyanin (KLH). KLH could be conjugated via a sulfydryl group - (Myers et al.)

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Mouse Monoclonal Antipeptide Antibodies Specific for Cholesteryl Ester Transfer Protein (CETP)

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ABSTRACT

A synthetic peptide whose amino acid sequence corresponds to residues 131–142 of human cholesteryl ester transfer protein (CETP) was used as an immunogen to generate a panel of monoclonal antibodies (MAbs) specific for the intact CETP molecule. Spleen cells from BALB/c mice immunized with the peptide conjugated with keyhole limpet hemocyanin (KLH) were fused with SP2/0 myeloma cells. Two MAbs that bound fixed peptide in an enzyme-linked immunoabsorbent assay (ELISA) were partially characterized regarding their specificity and biological activity. ATM192 of the IgG_1 subclass and J16-14 of the IgG_3 subclass were used in a Western blot assay as well as in the ELISA. We have also shown through the use of immunoprecipitation that ATM192 can remove CETP enzyme activity from human serum without destroying the enzyme's activity. We have also shown that the antibodies can bind CETP from rabbits. The specificity studies and the lack of inhibition of enzymatic activity suggest that the MAbs bind a structural area of the CETP molecule not a part of the active binding site of the enzyme. We conclude that these antibodies can be valuable as tools for studying CETP levels in human serum as well as in tissue homogenates from rabbits and humans.

INTRODUCTION

CHOLESTERYL ESTER TRANSFER PROTEIN (CETP), a plasma enzyme, is a hydrophobic glycoprotein responsible for the facilitated transfer and exchange of neutral lipids such as cholesterol ester and triglycerides between lipoproteins, therefore it plays an important role in reverse cholesterol transport.[1] The human form of the enzyme CETP has been purified and the amino acid sequence determined.[2] The scheme used to purify the enzyme has proved difficult due to the hydrophobic nature of the enzyme.[3] Few have been able to produce an antibody able to quantify CETP mass,[4] although neutralizing monoclonal antibodies are available.[5] The objective of this study was the preparation of mouse monoclonal antibodies against a synthetic peptide derived from the published amino acid sequence of the human CETP molecule. The amino acid motif chosen as the immunogen was from an area removed from the active binding site of the enzyme. We have produced several MAbs that are specific for epitopes found on the peptide as well as the intact molecule. Two of these MAbs have been partially characterized and their use in established assays are described.

MATERIALS AND METHODS

Animals

Female BALB/c mice were obtained from the East Carolina University School of Medicine's department of Comparative Medicine animal facility. The mice were fed mouse chow and water *ad libitum*. The experimental animals were maintained and treated following approved guidelines as described by the U.S. Department of Agriculture and the National Institutes of Health. All protocols were approved by the East Carolina University Animal Care and Welfare Committee.

Generation of anti-CETP antibodies

The 12-mer CETP peptide (CETP 12-mer), N-Cys-Asp-Ser-Gly-Arg-Val-Arg-Thr-Asp-Ala-Pro-Asp-C, corresponding to amino acids 131–142 of the CETP sequence, was synthesized (Dr. David Klapper, UNC-Chapel Hill, Department of Microbiology and Immunology) and conjugated to keyhole limpet hemocyanin (KLH). The CETP 12-mer-KHL conjugate

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(CETP 12-mer-KLH) was dissolved in sterile saline and emulsified in Freund's complete adjuvant. Each mouse received 20 μg of the conjugate injected intraperitoneally (ip). A second injection of 25 μg of CETP 12-mer-KLH in Freund's incomplete adjuvant was administered ip; 24 days later a final injection of 20 μg in sterile saline was given subcutaneously (sc) 31 days after the initial injection. The mice were tested for antibody activity 7 days after the final injection and were subsequently boosted ip with 20 μg of CETP 12-mer-KLH in sterile saline 3 days before fusion.

Spleens were obtained from 2 mice with high antipeptide serum titers and single-cell suspensions were made in serum-free Ultradoma (Sigma Chemical Co., St. Louis, MO). Spleen cells and SP2/o myeloma cells were mixed and pelleted at a ratio of approximately 5:1 (spleen cells to myeloma cells). The fusion was accomplished using the following protocol performed routinely in this laboratory. The cell pellet was gently loosened while adding 1.5 ml of polyethylene glycol (PEG) 3500 (Sigma Chemical Company, St. Louis, MO) dropwise over 45–60 sec. Serum-free, antibiotic-free Ultradoma medium was immediately added as follows: 3 ml over 30 sec, 9 ml over 30 sec, and 3 ml over 30 sec. The cells were then incubated for 8 min at room temperature followed by 2 min at 37°C, then centrifuged at 200*g* for 5 min. The pellet was resuspended in 200 ml of hypoxanthine–aminopterin–thymidine medium (HAT) and plated into 96-well microtiter plates (Corning, NY) at 0.2 ml/well. Peritoneal macrophages from BALB/c mice were added to each well at a concentration of 5×10^3 cells/well as a feeder layer.

Wells containing MAb specific for the peptide were identified by ELISA as described below and the hybridomas cloned by limiting dilution in Ultradoma containing 10% iron-supplemented new born calf serum (Sigma Chemical Co., St. Louis, MO) with 10% supernate from the American Type Culture Collection macrophage line P388D1.

Two lines were selected for propagation in 2,6,10,14-tetramethylpentadecane (Pristane, Sigma Chemical Co., St. Louis, MO) primed BALB/c mice from which ascites fluid was subsequently collected. The MAbs were purified using the E-Z-SEP system (Middlesex Sciences, Foxborough, MA).

ELISA

Immune sera were tested for anti-CETP 12-mer activity using a solid phase ELISA. CETP 12-mer was coupled to a Covalink plate (Covalink, NUNC, Roskilde, Denmark) exactly as described by the manufacturer. The peptide was dissolved in distilled-deionized water (ddH_2O) at a concentration of 2 mg/ml. Peptide activation was achieved by the addition of 50 μl of the peptide + 50 μl *N*-hydroxysuccinimide (NHS) + 1-ethyl-3-(3-dimethyl aminopropyl)carbodimide (EDC), 0.1 M each dissolved in ddH_2O water for exactly 30 min at room temperature. Ice cold 0.1 M carbonate buffer, pH 8.6, was added to give a final concentration of 1 μg/well, which was incubated 30 min at 4°C. Following coupling of the peptide, the plate was washed three times for 10 min per wash with Cova buffer, then allowed to stand for a further 15 min in the final wash. Unreacted binding sites on the plate were blocked with 0.5% bovine serum albumin (BSA) in 0.1 M carbonate buffer, pH 8.6, for 40 min at room temperature. Following blocking, 100 μl/well of master well supernatant was incubated at 4°C overnight. Subsequently, the plates were washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-Tween), followed by the addition of 100 μl of biotinylated antimouse IgG (Sigma Chemical Co., St. Louis, MO) in PBS-Tween, and incubated for 30 min at 37°C. Following three additional 10 min washes in PBS-Tween, streptavidin-alkaline phosphatase (Zymed, San Francisco, CA) was added to the wells and incubated a further 30 min. Finally, three 10 min washes in PBS-Tween were followed by the addition of Sigma-104 alkaline phosphatase substrate (Sigma Chemical Co., St. Louis, MO). The 96 well plates were read at a wavelength of A_{405nm} in an Anthos microplate plate reader (Denley Instruments Inc., Durham, NC).

Antibody competition assays

Estimation of avidity and epitope binding of the MAb ATM192 was done by competitive assays that competed free CETP 12-mer with peptide immobilized on Covalink plates, for the binding by the MAb. Varying concentrations of synthetic peptide were mixed in equal volumes with a predetermined limiting dilution of MAb and incubated for a period of 1 h at 37°C. The antigen-antibody mixture was then transferred to individual wells containing 1 μg/well peptide, and incubated overnight at 4°C. The presence of ATM192 was detected by the addition of biotinylated antimouse IgG (Sigma Chemical Co., St. Louis, MO) as described under ELISA.

Varying concentrations of purified ATM192 were also competed with polyvalent rabbit anti-CETP whose preparation has been previously described.[6] ATM192 was incubated in a Covalink plate containing bound peptide at 1 μg/well for 1 h at 37°C. Equivalent volumes of purified rabbit antiserum at a limiting dilution of 4 ng/well were added to the wells and the reaction mixture was incubated for an additional 2 h at 37°C then overnight at 4°C. The presence of rabbit antipeptide antibody was detected by the addition of biotinylated antirabbit IgG (Sigma Chemical Co., St. Louis, MO) as described under ELISA.

The relative binding avidities of ATM192 and J16-14 were compared by competing the two against a fixed amount of peptide. Various concentrations of ATM192 were incubated with 1 μg of CETP 12-mer fixed in Covalink plates for 1 h at 37°C followed by the addition of 300 ng of J16-14 for an additional 2 h. The assay was developed as above by using IgG_1-specific, biotinylated antibody.

CETP preparation

Cholesteryl ester transfer protein was used either as a semipurified preparation or from human and rabbit liver homogenates. CETP was semipurified from human plasma obtained from the blood bank at Pitt Memorial Hospital, Greenville, NC. The procedure essentially involved a combination of ultracentrifugation and hydrophobic interaction chromatography on phenyl-Sepharose as described by Tollefson and Albers.[7] Biopsy samples from human liver and whole livers from New Zealand White rabbits were homogenized as previously described.[8] The supernatants from the liver preparations and CETP preparations from human plasma were stored frozen at −20°C until used.

Immunodot-blot

Nitrocellulose membranes (Transphor™, Hoeffer Scientific Instruments, San Francisco CA) were used in a dot-blot apparatus (Bio-Rad, Hercules, CA). Preparations of semipurified CETP (approx. 1 μg/ml) were loaded into wells and incubated at room temperature for 1 h then light suction was applied to draw the remaining liquid through the membrane. The membrane was washed three times with PBS, then blocked overnight at 4°C with 1% dry milk in PBS. Following blocking, the membrane was immersed in ATM192 at a concentration of 25 μg/ml and incubated for 1 h at room temperature. ATM192 binding was detected by the use of the enhanced chemiluminescence system as described under Western blot analysis.

Western blot analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described by Laemmli.[9] Preparations of human serum, semipurified CETP, and homogenates of human and rabbit liver were diluted in sample buffer (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol). The samples were heated in a boiling water bath for 5 min prior to loading on to an SDS–12%PAGE Mighty Small I™ vertical slab gel (Hoefer Scientific Instruments, San Francisco, CA). Prestained low range SDS-PAGE standards (Bio-Rad, Hercules, CA) were run in a parallel lane for size estimation. Protein transfer onto Immobilon-P membranes (Millipore, Bedford, MA) was done according to the method of Towbin *et al.*,[10] then unbound sites on the membrane were blocked with 1% BSA in PBS overnight at 4°C.

Immunoreactive bands were identified using Western Light chemiluminescence (Tropix, Inc., Bedford, MA) with slight modifications. The membrane was flooded with either ATM192 or J16-14 at a concentration of 25 μg/ml then incubated at room temperature for 1 h. The membrane was washed using the following procedure: two 5 min washes in PBS-Tween-20 (Tropix wash buffer), two 5 min washes in PBS + 3% Tween-20, then two 5 min washes in Tropix blocking buffer. The remaining detection procedure was performed as described by the manufacturer.

Specificity of ATM192

CETP was purified as described above (CETP). Semipurified CETP was assayed for enzyme activity as described.[7] The enzyme activity purified from individual patients was normalized to 100%.

Biological specificity of ATM192 ws determined by the removal of CETP activity by the MAb. The CETP was incubated with 0.5 μg of purified ATM192 at a 1:1 ratio for 1 h rotating at 37°C. To the antibody-CETP complex, 0.25 ml of antimouse IgG agarose (Sigma Chemicals, St. Louis, MO) was added and the reaction mixtures incubated as before. Finally the mixtures were microcentrifuged and the supernatants extracted then analyzed for CETP enzyme activity.

ELISAs confirming the specificity of ATM192 for CETP 12-mer versus nonspecific interactions were performed using an irrelevant 35-mer peptide (c-erbAβ1-pep[11]) as a negative control. ATM192 was incubated with the CETP 12-mer in a Covalink plate as described under ELISA. In addition, c-erbAβ1-pep was conjugated to a Covalink plate and incubated with ATM192 also. A c-erbAβ1-pep-specific IgG_1 MAb (β36[11]) was used as a positive control for binding to the irrelevant peptide.

Antigen capture assay

Purified ATM192 was diluted in carbonate buffer, pH 9.6, and added to EIA/RIA strip plate (Costar, Cambridge, MA) to a final concentration of 100 ng/well. The wells were incubated overnight at 4°C. Following coupling of the MAb, the solution was removed and the plates were filled with 0.3 ml of 5% bovine serum albumin (BSA) in 0.1 M carbonate buffer pH 9.6 for 1 h at 37°C. After blocking, the wells were washed for 10 min with PBS containing 0.05% Tween 20 (PBS-T). Human serum samples to be quantified were added in 100-μl aliquots. The serum samples were used either untreated or treated with 0.5% SDS as described by Sato *et al.*[12] The plates were incubated for 2 h at 37°C and subsequently washed with PBS-T. Polyclonal IgG rabbit anti-CETP antibody at 5 μg/ml in PBS-Tween was then added to the wells for 1 h. The plates were then washed with PBS-T for 10 min. After washing, 100 μl of biotinylated antirabbit IgG (Sigma Chemicals, St. Louis, MO) at a dilution of 1:1000 in PBS-T was pipetted into each well and incubated for 30 min at 37°C. Following a 10 min PBS-T wash, strepavidin-alkaline phosphatase (Zymed, San Francisco, CA) was added at a working dilution of 1:1000 in PBS-T at 100 μl/well and incubated a further 30 min. A final 30 min wash in PBS-T was done followed by the addition of Sigma-104 alkaline phosphatase substrate (Sigma Chemical Co., St. Louis, MO). Optical density was then recorded (OD_{405nm}) in an Anthos microplate plate reader (Denley Instruments Inc., Durham, NC).

RESULTS

Selection of hybridomas

Three BALB/c mice were immunized with the synthetic peptide (residues 131–142) derived from the CETP molecule. Following the last injection of the immunization protocol, the sera were tested by ELISA for binding activity against both the peptide and semipurified CETP. Two of the mice having identical serum titers were used for the preparation of spleen cells for fusion. There was growth in 90% of the culture wells. After selection and multiple clonings, two MAbs were selected for study; ATM192, an $IgG_{1(\kappa)}$ and J14-16, an $IgG_{3(\kappa)}$. Third generation ascitic fluid containing the mAbs had $\log_2$ titers of ≥ 106.

Specificity of ATM192

It was necessary to determine the specificity of the ATM192 and this was done first by determining the biological specificity, and then by comparing binding characteristics of the MAb with other irrelevant antibodies and antigens.

It was found that MAb ATM192 had no effect on the biological activity of CETP as the MAb did not significantly reduce the enzymatic activity (Table 1). If, after reacting the semi-

TABLE 1. THE EFFECT OF ATM192 ON THE BIOLOGICAL ACTIVITY OF CETP REACTANTS

Reagents	*Activity (%)*
Ag[a]	100
Ag + MAb[b]	77
Ag + MAb + antimouse IgG-agarose	0
Ag + antimouse IgG-agarose	73

[a]CETP.
[b]ATM192.

purified enzyme with the MAb, the reaction mixture was absorbed with Sepharose-anti-IgG, all enzymatic activity was lost. These data suggest that ATM192 binds the intact native protein in an area removed from the active site of the enzyme.

To further confirm the specificity of ATM192 for CETP, CETP 12-mer of c-erbAβ1-pep were immobilized onto a Covalink plate as described in Materials and Methods (ELISA), then either ATM192 or β36 were assessed for binding as before (Table 2). As expected, β36 bound strongly to c-erbAβ1-pep, whereas ATM192 did not. In the reciprocal experiment, ATM192 strongly bound CETP 12-mer while β36 did not generate an OD_{405nm} greater than background. A number of MAbs with specificities irrelevent to this report were assayed for CETP 12-mer binding which also failed to generate ODs above 1% that of ATM192. These data infer that nonspecific interactions were not involved in ATM192 binding to the CETP 12-mer nor the native protein.

These collective data support the conclusion that ATM192 is highly specific both for native CETP and the synthetic 12-mer.

Immunoblot analysis

A pilot experiment was done in which various concentration of semipurified CETP were spotted onto nitrocellulose membrane then incubated with a constant amount of ATM192 and developed using chemiluminescence (Fig. 1). It was found that the MAb readily bound the CETP and the strength of signal generated was concentration dependent. Similar results were observed when J16-14 (IgG_3) was used as the primary antibody (data not shown).

The ability of ATM192 and J16-14 to bind various preparations of SDS-PAGE separated, electroblotted CETP was also studied. Subsequent to transfer and chemiluminescent detection with ATM192, CETP appeared as a band of approximately 70 kDa (Fig. 2). The concentration of CETP in the various preparations was not determined, but a major CETP band

TABLE 2. ATM192 BINDS SPECIFICALLY TO THE CETP 12-MER PEPTIDE IN ELISA

MAb	*c-erbAβ1-pep*	*CETP 12-mer*
ATM192	0.1[a]	1.0
β36	1.56	<0.01

[a]All absorbance values (OD_{405nm}) represent the average of duplicate assays.

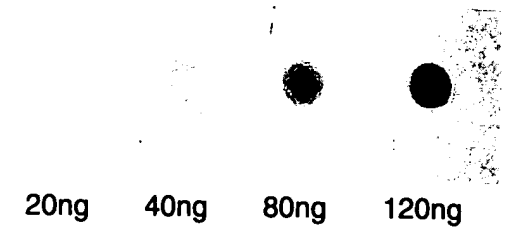

FIG. 1. Detection of CETP by Immuno Dot Blot using ATM192. Reactivity of 25 μg/ml of ATM192 with *1.* 20ng, *2.* 40ng, *3.* 80ng, *4.* 120ng of semi-purified CETP. ATM192 binding was detected using chemiluminescence.

was consistently visible using an optimal amount of purified MAb. Under the same conditions we observed that liver preparations contained a higher level of CETP and that both ATM192 and J16-14 detect CETP in human preparations.

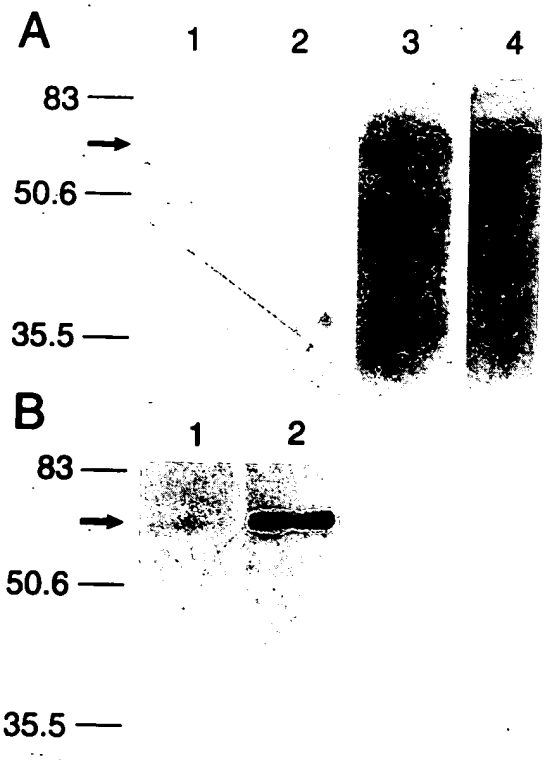

FIG. 2. Western Blot Analysis of CETP Content in Human Plasma, Human Liver and Rabbit Liver Preparations using mAbs ATM192 and J16-14 as Probes. (A) Bands were developed using 25mg/ml ATM192. Lane 1: semi-purified CETP; lane 2: CETP in human plasma; lane 3: CETP in human liver; lane 4: CETP in rabbit liver. (B) Bands were developed with 25mg/ml J16-14. Lane 1: semi-purified CETP; lane 2; CETP in human liver. Approximate molecular weights (kDa) and the CETP signal are indicated.

Furthermore, it was also noted that ATM192 reacted with CETP in rabbit liver preparations (Fig. 2) demonstrating close epitope homology at the amino acid level between the two species.

Competitive studies

The avidity of ATM192 and J16-14 for CETP 12-mer was estimated by ELISA (Fig. 3). Limiting dilutions of J16-14 were used to compete with ATM192 for binding to fixed CETP 12-mer (Fig. 3A). Approximately 2 μg of purified ATM192 was required to inhibit 50% binding of 300 ng of J16-14 to the 12-mer. These data suggest that J16-14 binds the synthetic CETP peptide with a greater avidity than ATM192. Further experiments were done in which 300 ng of ATM192 was competed with free CETP 12-mer and 1 μg of fixed peptide. It was found, that 4 ng of free peptide could inhibit 50% binding to the 1 μg of fixed peptide (data not shown). This result would support the conclusion that ATM192 binds the peptide antigen with a reasonably high avidity.

It was of further interest to determine whether there were dominant epitopes associated with the 12-mer used for immunization. A CETP-specific rabbit polyclonal antibody[6] was incubated with ATM192 to compete for binding to 1 μg of immobilized CETP 12-mer. It was found that 75 ng of ATM192 generated 50% inhibition of binding by 4 ng of the rabbit antibody to 1 μg of the fixed peptide (Fig. 3B).

From these data, we concluded that there was a dominant epitope recognized by both populations of antibodies. Since the rabbit antibody was from a hyperimmunized animal, it can be assumed that the ATM192 was competing with a spectrum of rabbit antibodies with high affinity for CETP. These data further support the conclusion that ATM192 binds the peptide with a reasonably high avidity.

CETP quantitation

ATM192 was used as a capture antibody to quantify CETP in human serum. Sato *et al.*[12] reported that the use of sodium dodecyl sulfate (SDS) increased the sensitivity of a capture assay in which an MAb was used to capture CETP. In preliminary experiments as a capture antibody in the ELISA format, ATM192 was found to efficiently bind CETP in human serum in the presence of SDS (Table 3). These experiments have shown that ATM192 as the capture antibody and the polyvalent rabbit antipeptide antibody as the second antibody can be used with a standard curve to quantify CETP in human serum.

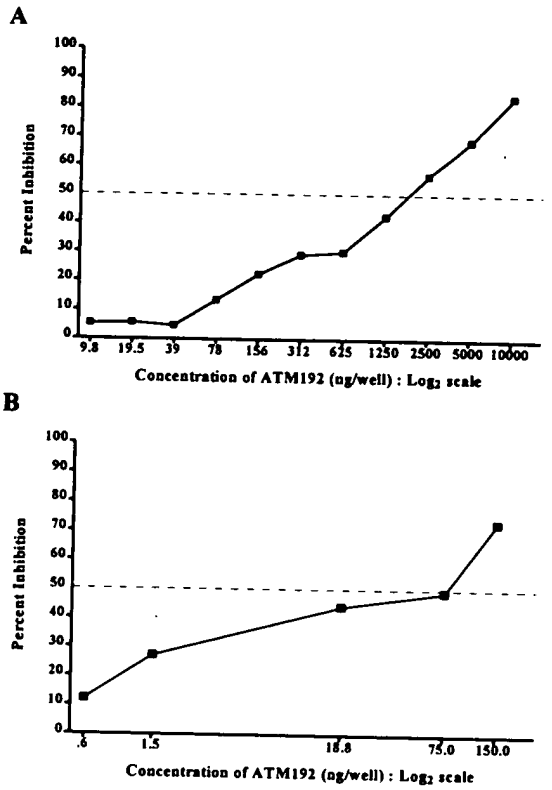

FIG. 3. A & B. Estimation of the Avidity of Anti-CETP mAb by ELISA. (A) 300 ng of J16-14 competes with ATM192 for the binding of 1 μg CETP peptide. (B) 4ng of polyclonal rabbit anti-CETP antibody competes with ATM192 for the binding of 1 μg of peptide. Dotted lines indicate 50% inhibition of binding. Data points represent the average of duplicate assays.

DISCUSSION

Published protocols for the purification of human cholesteryl ester transfer protein were found to be complex and difficult when attempting to obtain sufficient quantities for immunization purposes. Consequently we used a synthetic peptide derived from the structure of the intact protein molecule as an immunogen to elicit antibodies in BALB/c mice. We have shown that two MAb, one an $IgG_{1\kappa}$ and the other an $IgG_{3\kappa}$, are functional antibodies capable of binding the native CETP protein in an area that does not interfere with the function of the enzyme.

TABLE 3. ATM192 USED AS A CAPTURE ANTIBODY IN A QUANTIFYING ELISA

CETP source[a]	*Without SDS*	*With SDS*[b]	*Increase in sensitivity (%)*
Serum pool A	0.664[c]	0.843	21
Serum pool B	0.361	1.583	57
Diabetic patient	0.191	1.281	85

[a]Each well contained 1 μg/ml of ATM192 as the capture antibody.
[b]SDS used at 0.5%.
[c]All absorbance values (OD_{405nm}) represent the average of duplicate assays.

In addition, these MAb were able to detect the enzyme in human serum as well as from homogenates of human and rabbit liver. Furthermore we have shown that the two MAb are specific for both the synthetic peptide and the native protein, binding with an adequate avidity. Based on our findings, we propose that the anti-CETP MAb can be used in experiments designed to quantify the enzyme as well those used to determine the effects of various drugs on its synthesis and utilization in humans.

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Antibodies to a synthetic peptide that react with flavin-containing monooxygenase (HLFMO3) in human hepatic microsomes.

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Flavin-containing monooxygenases (FMOs) catalyze the oxidation of a diverse array of xenobiotic compounds. The purpose of this investigation was to develop a specific immunological probe to human hepatic flavin-containing monooxygenase (HLFMO3). An oligopetide corresponding to amino acid residues 257-270 of HLFMO3 was coupled to **keyhole limpet hemocyanin** (KLH) through the sulfhydryl group of a cysteine residue added to the **amino-terminus** of the peptide. This peptide-KLH **conjugate** was used to generate a polyclonal antibody. The resulting immunoglobulin showed specific Western blot reactivity with HLFMO3 protein in human hepatic microsomes, the same protein that is recognized by a polyclonal antibody directed against macaque liver FMO. These findings demonstrate that an antibody directed against a synthetic peptide derived from HLFMO3 can be easily produced in large quantities and used in studies for the immunodetection and immunoquantification of HLFMO3. This is also the first antipeptide antibody directed against an FMO of any species.

... HLFMO3). An oligopetide corresponding to amino acid residues 257-270 of HLFMO3 was coupled to **keyhole limpet hemocyanin** (KLH) through the sulfhydryl group of a cysteine residue added to the **amino**

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ANTIBODIES TO HUMAN INTERLEUKIN-2 INDUCED BY SYNTHETIC POLYPEPTIDES
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Abstract:
Chemically synthesized polypeptides containing about 6 to about 40 amino acid residues having amino acid residue sequences that substantially correspond to the amino acid residue sequences of antigenic determinants of interleukin-2, when administered alone or as polymers or as **conjugates** bound to carriers, induce the production of antibodies of predetermined specificities. The polypeptides and the antibodies produced thereto can be used in diagnostic systems to measure the presence and amount of interleukin-2 and interleukin-2 cell surface receptors or binding sites in an assayed sample.

Abstract: